of monomeric NS3 helicase. The model is based on structural, biochemical and single molecule measurements of the helicase. First, we have assumed that NS3 helicase walks unidirectionally by alternately moving its two translocase domains forward one nucleotide (nt) at a time, as that being suggested for a similar helicase PcrA. Second, in our model NS3 helicase displays diffusive character upon ATP binding (before helicase dissociation), due to weak affinity of the helicase to the ssDNA. The helicase diffusion leads to occasional backward steps and increases displacement fluctuations that are supposedly detectable from single molecule experiments. Further, our model suggests that NS3 helicase interacts with the duplex at the ss-ds junction such that (i) the helicase can actively unwind the duplex by reducing free energy of base pairing /stacking at the duplex end; (ii) the helicase can be stabilized at the junction by its favorable association with the duplex backbone. By fitting with NS3 unwinding data from single molecule optical-tweezer measurements, our model explains sequence dependences of the unwinding velocity, efficiency, and helicase dissociation rate. In particular, based on the experimental data, we have quantified how active the NS3 helicase is in its unwinding, and estimated how fast the helicase diffuses in its ATP bound state. The generic features of coupling ssDNA/RNA translocation with duplex unwinding presented in our NS3 helicase model may apply as well to other similar systems.

1915-MiniSymp

The RSC Chromatin Remodeling ATPase can Translocate DNA with High Force and Small Step Size

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ATP-dependent chromatin remodeling complexes (remodelers) use the energy of ATP hydrolysis to reposition and reconfigure nucleosomes. Despite their diverse functions, all remodelers share highly conserved catalytic ATPase domains, many of which are shown to translocate DNA. Understanding remodeling requires biophysical knowledge of the DNA translocation process: how the ATPase moves DNA and generates force, and how translocation and force generation are coupled on nucleosomes. Here we characterize the real-time activity of a minimal translocase 'motor' complex isolated from a prototypical remodeler (RSC) on bare DNA, using high-resolution optical tweezers and a 'tethered' translocase system. We observe on dsDNA a processivity of ~35 bp, a speed of ~25 bp/sec, and a step size of 1.9 ($\pm$ 0.3, s.d.) bp. Surprisingly, the motor is capable of moving against high force, up to 30 pN, making it one of the most force-resistant motors known. We also provide evidence for DNA 'buckling' at initiation. These observations extend and clarify measurements of nucleosome-dependent translocation by the complete RSC or SWI/SNF complex, and reveal the ATPase as a powerful and versatile DNA translocating motor capable of disrupting DNA-histone interactions by mechanical force using a small step size.

1916-MiniSymp

The Enzymatic Motor Activity of Nonmuscle Myosin II-B is not Critical for Cardiac Myocyte Cytokinesis

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Nonmuscle myosin (NM) II is believed to drive contractile ring constriction during cytokinesis and NM II-B is the major nonmuscle myosin II isoform expressed in cardiac myocytes. Ablation of NM II-B in mice resulted in premature binucleation in cardiac myocytes consistent with a role for NM II-B in cytokinesis. Surprisingly binucleation can be rescued by expressing a motor-impaired mutant form of NM II-B (R709C) in the mouse heart, suggesting that NM II-B enzymatic motor activity is not essential for cytokinesis. We tested this hypothesis directly by using a COS-7 cell line which resembles cardiac myocytes in containing only NM II-B and small quantities of NM II-C (<7%) but no NM II-A. Previous work had shown that using siRNA to deplete NMHC II-B in this cell line resulted in multinucleation due to a failure in cytokinesis. We now show that COS-7 cell multinucleation can be rescued by transfecting with mutant forms of NM II-B (R709C) or NM II-A (N93K). These two mutant NM IIs have previously been shown to have marked reductions in their actin-activated MgATPase activity (70% in the case of NM II-B and 96% in the case of NM II-A) and neither could propel actin filaments in an *in vitro* motility assay. Importantly, cytokinesis is inhibited by the myosin II inhibitor blebbistatin in COS-7 cells expressing either wild-type NM II-B or the mutant form of NM II-A (or II-B). Whereas blebbistatin blocks myosin in an actin-detached state, the mutant NM IIs (R709C II-B and N93K II-A) have no defect in their actin-binding activity. Our results therefore argue that the role of NM II in cytokinesis depends more on its actin binding (cross-linking) property than its enzymatic Mg^{2+}-ATPase activity.

1917-MiniSymp

Dynein's Dual Roles as a Motor and Tether in the Neuron

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While the myosin and kinesin superfamilies achieve diverse functions through a large number of motors, each specialized to its role, cytoplasmic dynein accomplishes varied roles in the cell through diverse interacting partners. We hypothesized that in addition to its role in bidirectional organelle transport, mammalian dynein might also play role in tethering microtubules at the cortex, analogous to the well-characterized function of dynein in S. cerevisiae. We identified a direct interaction between dynein and the neuronal cell adhesion molecule NCAM180 that recruits dynein to the cortex. To test the ability of dynein to tether microtubule plus ends, microtubules containing short biotinylated segments at the minus end were attached to the coverslip, allowing the non-biotinylated plus-ends to exhibit diffusive movements. When dynein-coated beads were brought near to the plus-end using an optical trap, the microtubule became tethered, while beads coated with BSA or kinesin-1 did not effectively restrict lateral fluctuations. In dynamic microtubule assays, plus-ends tended to associate with dynein-coated beads and continue to grow, remaining in contact with a bead for 94+/-18 s (mean+/-SEM). In contrast, encounters between growing plus-ends and BSA-coated beads often led to catastrophe, decreasing the contact time to 45+/-16 s. In live cell assays we also measured a significant increase in microtubule dwell time in the vicinity of NCAM patches (189+/-12 s, compared to 100+/-7 s in controls). Together, these data support a mechanism in which dynein is anchored to the cell cortex by NCAM180 and transiently tethers the plus-ends of microtubules. Tethered microtubules likely provide a direct connection between the cell center and sites of cell-cell interaction, either mechanically or as preferred routes for transport, creating a powerful mechanism for cells to respond to or produce stimuli in the extracellular environment.

1918-MiniSymp

Kinesin-1/Hsc70-Dependent Mechanism of Slow Axonal Transport and its Relation to Fast Axonal Transport

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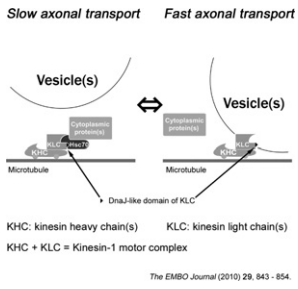
Cytoplasmic protein transport in axons ('slow axonal transport') is essential for neuronal homeostasis, and involves Kinesin-1, the same motor for membranous organelle transport ('fast axonal transport'). However, both molecular mechanisms of slow axonal transport and difference in usage of Kinesin-1 between slow and fast axonal transport have been elusive. Here, we show that slow axonal transport depends on the interaction between the DnaJ-like domain of the kinesin light chain in the Kinesin-1 motor complex and Hsc70, scaffolding between cytoplasmic proteins and Kinesin-1. The domain is within the tetratricopeptide repeat, which can bind to membranous organelles, and competitive perturbation of the domain in squid giant axons disrupted cytoplasmic protein transport and reinforced membranous organelle transport, indicating that this domain might have a function as a switchover system between slow and fast transport by Hsc70. Transgenic mice overexpressing a dominant-negative form of the domain showed delayed slow transport, accelerated fast transport and optic axonopathy without elevation of intraocular pressure. These findings provide a basis for the regulatory mechanism of intracellular transport and its intriguing implication in the understanding of neuronal dysfunction such as normal tension glaucoma.



Platform AM: Imaging & Optical Microscopy

1919-Plat

Compact Multi-Color STED Microscopy

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Stimulated emission depletion (STED) microscopy enables fluorescence imaging on the nanoscale with resolutions of 20 nm and beyond. As samples prepared for far-field fluorescence microscopy are in general also suitable for STED imaging, it has been applied advantageously in biosciences, in particular investigations of subcellular structures and assemblies.